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Influence of Certain Environmental Factors in the Field on Infection of Rice by Piricularia oryzae

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ABSTRACT

The amount of dew, the length of the dew period, and the leaf area at the time of inoculation were the only significant variables correlated with the average number of lesions per plant when rice (*Oryza sativa*) in the susceptible stage was inoculated daily during an 11-week period with conidia of *Piricularia oryzae*. Daily records were kept on dew, rain, temperature and relative humidity of the air, light, soil temperature, and leaf area of the rice plants. No apparent effect of air temperature in the range of 61-95 F on infection by the blast organism was found.

Experiments under controlled conditions have demonstrated the influence of temperature and moisture on infection of rice (*Oryza sativa* L.) by *Piricularia oryzae* Cav., the organism causing rice blast. Hemmi and Abe (5) found that the following minimal periods of continuous wetting were necessary to produce leaf infection: 19 hr at 90 F, 8 hr at 82 F, 6 hr at 75 F, and 6-8 hr at 68 F. Inoculated seedlings kept for 24 hr at 92 and 100% relative humidity by Abe (2) showed typical blast symptoms; those kept at 90% were healthy. Hashioka (4) reported that invasion and disease development take place most easily at 79-82 F and fairly readily at 66 or 91 F. Kahn and Libby (6) found that the optimum for infection was 80 or 85 F with 16-20 hr of dew.

Several interactions may negate the effect of factors in the field that have been demonstrated singly to be important under controlled conditions. In nature, high humidity or dew formation at night is generally a result of a considerable diurnal fluctuation in temperature. If high humidity or dew is necessary for infection, it might appear that infection is favored by low temperature because dew may not be formed at higher temperatures.

The temperature under which rice is grown affects its susceptibility to rice blast. Abe (1) found that the incidence of disease was lowest in seedlings grown at a soil temperature of 82 F and highest in those grown at 68 F. Hashioka (4) reported that resistance increases with the rise of both air and soil temperatures. The ratio of carbon to nitrogen in the leaves is increased in proportion to the rise in temperature.

Suzuki (7) inoculated the panicles of two resistant and two susceptible rice varieties grown at low and normal soil temperatures. Disease development in all plants in the low-temperature series was 75-100%, whereas at the normal temperature it was 0-13.3% for the resistant and 20-33.3% for the susceptible varieties.

Because of the effect of growth temperature on susceptibility, more infections may occur in the field when temperatures are low. Hashioka (4) stated that the severity of blast in the cool first-crop season in the tropical and subtropical regions and the comparative freedom from disease of the second crop are probably due to this factor.

The objective of this study was to determine the influence of certain environmental factors in the field on blast infection of rice and the interactions involved.

MATERIALS AND METHODS.—The rice breeding nursery at the Rice-Pasture Experiment Station, Beaumont, Texas, was the experimental site. It is in the commercial rice-growing area, where the culture of rice plants minimized interference of agronomic problems with research effort. The amount of natural blast infection in the Beaumont area is normally small, and the interference of naturally occurring inoculum with experimental inoculation can be avoided. The climate of the Beaumont area is such that a certain amount of variation in climatic conditions from day to day can be expected.

The six main plots each covered approximately 0.1 acre (40 × 90 ft). Each main plot was divided into 15 subplots, each 6 × 40 ft. Subplots were divided into eight sub-subplots, 5 × 6 ft. A 10-ft² area, 36 × 40 inches, within each sub-subplot was used for experimental work.

The rice variety C.I. 8970, used in our experiments, remained susceptible to blast over a relatively long period of growth. Main-plot plantings were staggered so that plants in a new main plot would be of sufficient size that they could be inoculated the day after the inoculations were completed on the previous main plot. Age of plants in the six main plots varied at the beginning of inoculations because of an increased rate of growth in the later-planted main plots, associated with more favorable growing conditions as the season progressed.

Seeds were planted with a nursery drill approximately 0.25 inch deep in rows with 9-inch spacings in harrowed soil. The plots were cultipacked after planting and flooded with about 2 inches of water. After about 24 hr the fields were drained, and they remained dry until after fertilizer had been applied. One hundred lb. of ammonium sulfate/main plot were applied broadcast 10-15 days after planting. The fields were flooded

after fertilization and remained flooded for the remainder of the experiment.

All inoculations were made with isolate 770 of race 3 of *P. oryzae*, obtained from Frances M. Latterell of these laboratories. Inoculation rates of 40.0, 4.0, 0.4, and 0.0 mg viable spores/10 ft² were used, with two replications of each rate each day. The range of rates of inoculum was selected to assure a sufficiently large number of lesions for reasonable accuracy in counting on days when climatic conditions were nearly limiting for lesion production. It also prevented an uncountably high number of lesions on all treatments when conditions for infection were favorable. The same relative position for each rate was used each day on all subplots.

Inoculations commenced daily at sundown. A spore and water suspension was introduced through 1.25-inch holes in the top center of 59-inch-high plastic inoculation tents that covered the 10 ft² areas to be inoculated. The inoculum was introduced into the tents by a DeVilbiss No. 152 atomizer nozzle attached to a No. 631 air gun powered by nitrogen gas at 25 psi from a commercial-type cylinder. The tents were placed over the sub-subplots just prior to inoculation and were allowed to remain in place until the spore aerosol appeared to have settled (usually about 20 min).

Fifteen subplots were inoculated in main plots 1 and 2, 13 in plot 3, 12 in plots 4 and 5, and 10 in plot 6. Sequential inoculations were made on consecutive subplots starting from one end of each main plot and proceeding each day toward the other end.

Plants from each 10-ft² inoculated plot and controls were allowed to remain in the field until the fourth day after inoculation. They were then pulled; the roots were washed and the plants taken to the laboratory where they were allowed to remain standing in water until the second day after pulling. This allowed the lesions to enlarge so that they would be easier to count. It also prevented sporulation of the lesions in the field. The second day after pulling, the total number of plants was determined and 50 plants were selected at random for lesion counts. Lesions were counted by examining each leaf on each of the 50 plants and determining the total number of lesions. This number was used to calculate the average number of lesions per plant.

Average plant size and approximate leaf area were determined at inoculation. Average size was determined by measurement of 20 plants for plant height and number of tillers. Approximate leaf area was determined from the mean approximate leaf area of two or three "average" plants; leaves from a single plant were placed side by side on the sticky side of a piece of tape and the area covered was measured.

Background assays for airborne spores were attempted with a sequential sampler (3). This technique was inadequate because of the unsatisfactory adhesive material used to coat the Rotobars. To determine their potential effect on infection, dew, wind, relative humidity, air temperature, soil temperature, rainfall, and light were measured.

The length of the dew period was measured by a dew meter of the type described by Taylor (8). Visual observations of the rate or amount of dew deposited were recorded. These observations were based on the width of the deposition line.

Both wind speed and wind direction were recorded continuously during the experiments with Signal Corps GMQ-1 signal generators attached to two Esterline-Angus AW recorders.

Relative humidity and air temperature were measured by hygrothermographs. Two instruments were located in shelters about 1 ft from the top of the levees at two locations in each main plot. A thermograph in the shelter measured the soil temperature. The probe was buried about an inch deep in the mud in the flooded paddies.

Rainfall was measured by an Instrument Corporation universal rain gage, which was calibrated once prior to installation in the field. It was placed on a platform at the edge of the field and was not moved during the experiments.

Only a relative measure of light intensity was obtained. This was done by use of a light integrator containing a photoelectric cell and a rectifier system. The counter shows a cumulative number of units of energy and thus indicates relative amounts of light energy from day to day but not units of light. The light integrator counter was read and zeroed each afternoon after the hours of bright light. Both units of energy and time that the instrument was read were recorded. In addition, visual observations of cloud cover were made daily.

RESULTS.—Lesion production, measured as the average number of lesions per plant, was 0-35.72 at 40 mg spores/10 ft²; 0-7.4 at 4 mg; 0-3.18 at 0.4 mg; and 0-2.70 at 0.0 mg (Table 1).

To use the analysis of variance technique, the data on the average number of lesions per plant were transformed to logs (number plus 0.01). Separate analyses were performed on the six main plots, and the plot variances were then tested for homogeneity by Bartlett's test. When plot variances were found to be uniform, it was possible to combine the six main plots for analysis to obtain over-all estimates of main effects and interactions with plot.

The effects of nine climatic or biological factors on lesion production were estimated by multiple linear regression analysis. These nine independent variates were (i) amount of dew, (ii) length of dew period, (iii) hours from inoculation to dew fall, (iv) light units on the day of inoculation, (v) light units on the day after inoculation, (vi) mean 24-hr temperature (F), (vii) mean night temperature (F) from 8 PM to 8 AM, (viii) wind speed at time of inoculation, and (ix) leaf area at inoculation. Separate analyses were performed for each inoculum rate. The model was the equation

$$Y_e = a + b_1X_1 + b_2X_2 + \ldots + b_pX_p$$

where there are p independent variables. The linear model proposed in this equation was only an approximation of the relationship between dependent and inde-

TABLE 1. Average number of lesions appearing on field-grown rice seedlings following inoculation with conidia of *Piricularia oryzae*

Plot	Dates of inoculation (1959)	Total plants examined[a]	Rate of inoculation (mg viable conidia/10 ft²)				Average		
			40	4	0.4	0	Dew period	Leaf area	Night temp
		no.	*Avg no. lesions/plant*				*hr*	*cm²*	*F*
1	30 April to 14 May	6,000	7.034	0.619	0.179	0.009	7.46	47.78	71.2
2	18 May to 1 June	6,000	7.304	0.699	0.137	0.078	9.72	49.39	72.5
3	2-14 June	5,200	4.541	0.757	0.456	0.433	9.98	40.45	73.6
4	15-26 June	4,800	1.447	0.157	0.072	0.045	8.25	38.82	77.4
5	27 June to 8 July	4,800	5.900	1.116	0.488	0.402	9.63	39.68	75.6
6	9-18 July	4,000	4.827	0.631	0.352	0.186	9.63	17.30	76.6

[a] This number of plants was equally divided among the four inoculum rate treatments.

pendent variables. For the range of values in this experiment, however, we thought that a linear model was sufficiently accurate for screening for the effective variates.

Results of these analyses are shown in Table 2. Values of R^2, the square of the multiple correlation coefficient, were on the order of 0.56 for all inoculum rates. R^2 represents the proportion of the variation in average number of lesions that is explained by the linear model given in the above equation.

Factors selected as important for lesion production were those shown to be significant at all inoculum rates: (i) amount of dew, (ii) length of dew period, and (iii) leaf area at the time of inoculation. Estimating equations were recomputed using three independent variates and are shown, with their 95% confidence limits for partial regression coefficients, in Table 3.

DISCUSSION.—The analysis of variance on climatic factors and certain variates indicates that some climatic factor or a combination of climatic factors with other variables affects numbers of lesions. Further validation is provided by the multiple linear regression analysis described previously.

Both length of dew period and amount of dew show high correlation with the number of lesions produced (Table 2). The importance of the amount of dew seems to be at odds with the findings of Abe (2), who found that infections occurred at 92 and 100% relative humidity, presumably in the absence of free water. The amount of dew in these studies was measured by a dew meter whose plate had maximum exposure to the sky in contrast to the varying exposure of rice leaves, and thus, it may not accurately reflect the presence of dew on the leaves. If the dew on the dew meter is heavy, the rice leaves are more apt to be completely covered with dew or surrounded by air of nearly 100% relative humidity. Thus, heavy dew on the meter may be a more accurate reflection of dew on the plants than light dew on the meter.

When dew was relatively heavy for about 8 hr or more, usually a considerable number of lesions were produced. When dew persisted for less than 8 hr or when there was no dew, few lesions were produced. When the quantity of dew was small even though the length of the dew period was about 8 hr or more, the number of lesions was generally well below that for a dew period of comparable length with a greater amount of dew. Five or more lesions/plant were produced 29 times during this study. In only two of these 29 cases was the dew quantity relatively small for a period of 8 hr or more. In the few cases in which there was relatively heavy dew for less than 8 hr, few lesions were produced.

The time from inoculation to the start of the dew period was positively correlated with lesion production for the inoculation rate of 0.4 mg spores/10 ft² (Table 2). This significant positive correlation is contrary to present concepts and to the results of a limited amount of previous work. Consequently, we have no explanation for this finding at present.

The data show no obvious effects of temperature on infection. The mean night temperature (average of recorded temperatures occurring at 2-hr intervals from 8 PM to 8 AM) ranged from 64.7 to 81.0 F during the test. Occasionally the mean night temperature fell below 65 F or rose above 78 F. Maximum day temperatures ranged from 74 to 95 F, with most days having a maximum of at least 80 F. Minimum night temperatures ranged from 61 to 75 F, with a majority of nights having minimums of 70 F or higher.

The partial correlation coefficients show that there is no statistical correlation between air temperatures and lesion number within the temperature range recorded, except between the mean 24-hr air temperature and the lesion number in the control plots. Perhaps this is an indication of a buildup of natural inoculum from a low level in the early spring to a much higher level as the season progressed and not necessarily a result of a beneficial effect of higher temperatures on lesion production. Thus, the levels of these two variables (temperature and natural inoculum) increased independently as the season progressed.

The data reveal no effects of relative humidity on initial infection. Relative humidity, as measured by hygrothermograph, reached 100% almost every night. The average length of the measured period of 100% relative humidity for the 77-day inoculation period was 10.04 hr. In most cases the measured period did

TABLE 2. Results of multiple linear regression analysis showing partial correlation and partial regression coefficients (and their standard errors) for the log (average number of lesions per plant +0.01) and each of the independent variates for which significance was found, arranged according to inoculum rate

Independent variate	0.0 mg/10 ft²		0.4 mg/10 ft²		4.0 mg/10 ft²		40 mg/10 ft²	
	Partial correlation coefficients (and their standard errors)							
Amount of dew (scale 1-8)	+0.27*	(0.118)	+0.28*	(0.117)	+0.37**	(0.110)	+0.45**	(0.101)
Length of dew period (hr)			+0.47**	(0.099)	+0.54**	(0.090)	+0.57**	(0.086)
Hr from inoculation to dew fall			+0.24*	(0.120)				
Mean 24-hr temp (F)	+0.54**	(0.090)						
Leaf area at inoculation (cm²)	+0.68**	(0.068)	+0.72**	(0.061)	+0.57**	(0.086)	+0.36**	(0.111)
	Partial regression coefficients (and their standard errors)							
Amount of dew (scale 1-8)	+0.057*	(0.024)	+0.047*	(0.020)	+0.081**	(0.026)	+0.13**	(0.032)
Length of dew period (hr)			+0.067**	(0.015)	+0.11**	(0.020)	+0.14**	(0.024)
Hr from inoculation to dew fall			+0.063*	(0.030)				
Mean 24-hr temp (F)	+0.11**	(0.021)						
Leaf area at inoculation (cm²)	+0.017**	(0.0022)	+0.017**	(0.0019)	+0.015**	(0.0025)	+0.0097**	(0.0030)

* Significant at the 0.05 level.
** Significant at the 0.01 level.

TABLE 3. Estimating equations for the combined effects of amount of dew, length of dew period, and amount of leaf area at time of inoculation on log (average number of lesions per plant plus 0.01) for the climate of Beaumont, Texas, and rice variety C.I. 8970 inoculated with isolate 770, race S, of *Piricularia oryzae* at four inoculum rates

Inoculum (mg/10 ft²)	Estimating equation[a]	Confidence limits (95%) for partial regression coefficients		
		X_1[b]	X_2[c]	X_3[d]
0.0	$Y = -2.3614 + .01304 X_1 + .04765 X_2 + .01520 X_3$	−.03816 to +.06424	+.00821 to +.08709	+.009824 to +.02058
0.4	$Y = -2.1974 + .003436 X_1 + .06753 X_2 + .01419 X_3$	−.05422 to +.04110	+.03853 to +.09653	+.01024 to +.01815
4.0	$Y = -2.3460 + .07117 X_1 + .1037 X_2 + .01284 X_3$	+.0025 to +.1121	+.07719 to +.14023	+.008537 to +.01714
40.0	$Y = -1.7344 + .1150 X_1 + .1321 X_2 + .008158 X_3$	+.07034 to +.1592	+.09808 to +.16608	+.003522 to +.01279

[a] Y = log (average no. lesions per plant plus 0.01), X_1 = amount of dew, X_2 = length of dew period in hours, and X_3 = leaf area at time of inoculation in cm².
[b] Values of dew amounts arbitrarily set at 8 for very heavy, 6 for heavy, 3 for light, and 1 for very light.
[c] Actual length of dew in hours is used in the equation.
[d] Actual value of leaf area in cm² is used in the equation.

not vary more than 1-2 hr from the mean. The start of the period of 100% relative humidity seldom varied greatly from day to day. Because of the relatively consistent day-to-day relative humidity, no effects of this factor on initial infection were noted.

We doubted the accuracy of the humidity-measuring device on the hygrothermograph used in our studies, but we have been unsuccessful in calibrating this device more accurately in the range of 95 to 100% relative humidity.

Generally, when there was a heavy or relatively heavy rain shortly after inoculation, few lesions appeared. When there was a sprinkle or a light shower after inoculation, some lesions appeared, but not as many as expected with a heavy dew of comparable length. It is possible that heavy or long rains washed spores from leaves.

There were too few instances of inoculations preceded by rain to establish any trends of the effects of this rainfall on lesion production. When this did occur, the number of lesions produced was relatively small, but at least one other condition considered important for infection was adverse.

An examination of the wind speed data indicates no direct influence on infection. This observation is based on a visual check of the range of wind speeds shown on the recorder charts. Average daily wind speeds were not calculated. Wind speed probably influenced initial infection indirectly by its effect on time of dew formation, length of the dew period, and amount of dew formed. The relationship of daily wind speeds and lesion production was not examined statistically. There was no statistical correlation between wind speed at inoculation and number of lesions.

The data indicated no significant effects of light on infection. However, the amount of natural infection appeared to be influenced by light. Following those periods when the sky was overcast or partially over-

cast for 1 or 2 days, the natural infection lesions seemed to be more abundant than during comparable periods of clear weather. Whether this apparent increase in the number of lesions in check plots was due to an increase in sporulation by the natural lesions in the field, was an indirect effect of factors associated with cloudy weather, such as an increase in length of dew period and slightly lower temperature, or was caused by some other factor is unknown.

In addition to the climatic factors, certain physiological and biological variables were evaluated for their effects on lesion production. These variables were leaf area of plants at inoculation, stage of growth of plants, and the effect of damage by rice water weevils.

The amount of plant leaf area increased with increasing plant age in all six main plots. Plants with greater leaf surface area also had more lesions. Leaf area at inoculation was statistically correlated with number of lesions. The effect of leaf area on lesion production was not considered for plants older than 37 days or for plants having more than 108 cm^2 of leaf surface.

The stage of growth and age of plants appeared to be closely but not completely related to leaf area. Leaf area increased with increasing age in all six main plots, but in only one of the four main plots where plants less than 20 days old were inoculated did any appreciable number of lesions appear, although in some cases, conditions were favorable for infection. This may have been due indirectly to exposed leaf area, which was considerably reduced by the flooded condition of the fields.

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